MANUFACTURING METHOD FOR SUBSTRATE ON WHICH NERVE CELLS ARE ARRANGED

BACKGROUND OF THE INVENTION

Field of the Invention

[0001] The present invention relates to a manufacturing method for a substrate on which nerve cells are arranged. Priority is claimed on Japanese Patent Application No. 2020-050906 filed in Japan on Mar. 23, 2020, Japanese Patent Application No. 2020-115635 filed in Japan on Jul. 3, 2020, and Japanese Patent Application No. 2020-217760 filed in Japan on Dec. 25, 2020, the content of which are incorporated herein by reference.

Description of Related Art

[0002] Nerve cells form a network in the living body and perform their actions in a functionally connected state. In the elucidation of the brain function, the toxicity evaluation of nerve system-involved disease, drug discovery and drug development, or the like, it is necessary to reproduce in vitro the activity state of nerve cells in the living body as accurately as possible, which is considered to be important for enhancing the extrapolation property of test results (the correlation with clinical data).

[0003] For this purpose, it is considered effective to develop a neural circuit model in which any nerve cells are arranged at predetermined positions and the nerve cells extend axons and are functionally connected to each other. However, it is difficult to precisely arrange nerve cells on a substrate by a manual procedure.

SUMMARY OF THE INVENTION

[0004] In Patent Document 1 (Japanese Unexamined Patent Application, First Publication No. 2019-162097), an attempt was made to arrange cells by using a pattern of a cell adhesive material and a cell non-adhesive material. However, the inventors of the present invention found that in a case where cells are arranged by using the pattern of a cell adhesive material and a cell non-adhesive material, it is difficult to cause cells to remain in a predetermined position since nerve cells in particular migrate in the cell adhesive portion. Therefore, an object of the present invention is to provide a technique for precisely arranging nerve cells on a substrate while suppressing the migration of nerve cells.

[0005] A manufacturing method for a substrate on which nerve cells are arranged, according to the present invention, includes a step of arranging, on a substrate, a plurality of liquid droplets containing nerve cells by an inkjet method to form one or a plurality of liquid pools, where the substrate has a region in which a cell adhesive material is arranged and a region in which a cell non-adhesive material is arranged; and a step of incubating the liquid pool until the nerve cells sediment and temporarily adhere onto the substrate to form a cell aggregate, where the diameter per one liquid pool is 500 μm or less and the density of nerve cells per one liquid pool is 10^5 cells/cm^2 or more.

[0006] According to the present invention, a technique for precisely arranging nerve cells on a substrate while suppressing the migration of nerve cells can be provided.

BRIEF DESCRIPTION OF THE DRAWINGS

[0007] FIG. **1** is a schematic view illustrating one example of an inkjet head.

[0008] FIG. **2** is a schematic view illustrating one example of an input waveform to an inkjet head.

[0009] FIG. **3** is a schematic view illustrating one example of an input waveform to an inkjet head.

[0010] FIG. **4** is a schematic view illustrating one example of a liquid droplet-arranging device.

[0011] FIG. **5** is a schematic view illustrating one example of a liquid droplet-arranging device.

[0012] FIG. **6** is a schematic view illustrating one example of a liquid droplet-arranging device.

[0013] FIG. **7** is a schematic view illustrating one example of a liquid droplet-arranging device.

[0014] FIG. **8** is a flow chart illustrating one example of a manufacturing method for a neural circuit model.

[0015] FIG. **9** is a schematic cross-sectional view illustrating a manufacturing method for a neural circuit model.

[0016] FIGS. **10**A to **10**C are photomicrographs taken in Experimental Example 1.

[0017] FIG. **11** is a graph showing the results of Experimental Example 2.

[0018] FIG. **12** is a schematic view illustrating a procedure for arranging a pattern of a cell non-adhesive material and a pattern of a cell adhesive material on a substrate and the arrangement of cells.

[0019] FIGS. **13**A to **13**D are photomicrographs taken in Experimental Example 3.

[0020] FIG. **14** is a fluorescence photomicrograph taken in Experimental Example 4.

[0021] FIGS. **15**A to **15**E are schematic views illustrating a procedure of Experimental Example 5.

[0022] FIGS. **16**A to **16**D are schematic views illustrating the arrangement of cells in Experimental Example 6.

[0023] FIG. **17**A is a schematic view illustrating an arrangement pattern of a cell non-adhesive material and nerve cells in a neural circuit model produced in Experimental Example 7. FIG. **17**B is a representative photomicrograph of a neural circuit model immediately after ejecting a cell ink in Experimental Example 7.

[0024] FIG. **18** is a fluorescence photomicrograph of a neural circuit model produced in Experimental Example 7.

[0025] FIGS. **19**A and **19**B are views illustrating a pattern of a cell non-adhesive material arranged on a substrate in Experimental Example 8.

[0026] FIGS. **20**A and **20**B are fluorescence photomicrographs taken in Experimental Example 8.

[0027] FIGS. **21**A and **21**B are photomicrographs taken in Experimental Example 9.

DETAILED DESCRIPTION OF THE INVENTION

[0028] Hereinafter, embodiments of the present invention will be described in detail with reference to the drawings as necessary. In the drawings, the same or corresponding parts are designated by the same or corresponding reference numerals, and the description thereof will not be duplicated. The dimensional ratio in each figure may be exaggerated for illustration and thus may not necessarily match the actual dimensional ratio.